

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

AMENDMENTS TO THE CLAIMS

This Listing of Claims will replace all prior versions, including listings, of claims in the application.

Listing of Claims

Claim 1 (previously presented): A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating pine cells of the *Pinus* subgenus with *Agrobacterium* for *Agrobacterium* transformation;

minimizing damage to cells subsequent to *Agrobacterium* infection by washing cells with a liquid culture medium comprising inorganic nutrients, vitamins, amino acids, a carbohydrate source and an *Agrobacterium* eradicant, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and

germinating said transgenic somatic embryos to produce transgenic plants.

Claim 2 (currently amended): The method of claim 1, wherein said damage to cells is minimized by:

(a) suspending cells having been incubated with *Agrobacterium* in a the liquid culture medium;

(b) agitating said liquid culture medium containing suspended cells to wash the cells and remove *Agrobacterium*; and

(c) recovering washed cells with minimal damage.

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 3 (previously presented): The method of claim 2, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

Claim 4 (currently amended): The method of claim 1, wherein said damage to cells is minimized by:

- (a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;
- (b) rinsing said cells using ~~a the~~ liquid culture medium to remove *Agrobacterium*; and
- (c) recovering washed cells with minimal damage.

Claim 5 (previously presented): The method of claim 4, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

Claim 6 (previously presented): The method of claim 4, wherein pine cells are plated onto a support membrane subsequent to *Agrobacterium* transformation.

Claim 7 (canceled).

Claim 8 (previously presented): The method of claim 4, wherein steps (b) and (c) are repeated between 2 and 10 times.

Claim 9 (previously presented): The method of claim 4, wherein each wash is carried out for a duration sufficient to expose all the cells to the liquid culture medium, said wash carried out for between half an hour to overnight in duration.

Claim 10 (canceled).

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 11 (original): The method of claim 4, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 12 (previously presented): The method of claim 1, wherein said selection is performed by

culturing washed cells with minimized damage on a support membrane placed over a gel medium;

contacting said cells with a selection agent; and
selecting transformed cells.

Claim 13 (original): The method of claim 12, wherein said selection agent is contained in said gel medium.

Claim 14 (original): The method of claim 12, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is placed on said gel medium.

Claim 15 (previously presented): The method of claim 14, wherein said layer is a layer of liquid medium.

Claim 16 (previously presented): The method of claim 14, wherein said layer is a layer of gelled medium.

Claim 17 (original): The method of claim 14, wherein said layer is a filter paper with a liquid medium absorbed therein.

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 18 (original): The method of claim 12, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 19 (previously presented): The method of claim 13 which further comprises contacting the washed cells with an *Agrobacterium* eradicant.

Claim 20 (previously presented): The method of claim 19, wherein the eradicant is contained in a layer in or positioned over the gel medium containing the selection agent.

Claim 21 (previously presented): The method of claim 20, wherein said layer containing the eradicant is a layer of liquid medium.

Claim 22 (previously presented): The method of claim 20, wherein said layer containing the eradicant is a layer of gelled medium.

Claim 23 (previously presented): The method of claim 20, wherein said layer containing the eradicant is a filter paper with a liquid medium absorbed therein.

Claim 24 (original): The method of claim 20, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 25 (previously presented): A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

incubating pine cells of the subgenus *Pinus* with *Agrobacterium* for *Agrobacterium* transformation;

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

minimizing damage to cells subsequent to *Agrobacterium* infection by washing cells with a liquid culture medium comprising inorganic nutrients, vitamins, amino acids, a carbohydrate source and an *Agrobacterium* eradicant, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells by culturing washed cells with minimized damage on a support membrane placed over a gel medium, contacting said cells with a selection agent and selecting transformed cells;

culturing said transformed cells to produce transgenic somatic embryos; and
germinating said transgenic somatic embryos to produce transgenic plants.

Claim 26 (previously presented): The method of claim 25, wherein said damage to cells is minimized by:

- (a) suspending cells having been incubated with *Agrobacterium* in a liquid culture medium;
- (b) agitating said liquid culture medium containing suspended cells to wash the cells and remove *Agrobacterium*; and
- (c) recovering washed cells with minimal damage.

Claim 27 (previously presented): The method of claim 26, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

Claim 28 (canceled).

Claim 29 (previously presented): The method of claim 26 which further comprises contacting the washed cells with an *Agrobacterium* eradicant during selection.

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 30 (previously presented): The method of claim 29, wherein the eradicant is contained in a layer in or positioned over the gel medium containing the selection agent.

Claim 31 (currently amended): The method of claim 25, wherein said damage to cells is minimized by:

- (a) plating pine cells having been incubated with *Agrobacterium* on a support membrane;
- (b) rinsing said cells using ~~a~~ the liquid culture medium to remove *Agrobacterium*; and
- (c) recovering washed cells with minimal damage.

Claim 32 (previously presented): The method of claim 31, wherein pine cells are plated onto a support membrane prior to *Agrobacterium* transformation.

Claim 33 (previously presented): The method of claim 31, wherein pine cells are plated onto a support membrane subsequent to *Agrobacterium* transformation.

Claim 34 (canceled).

Claim 35 (previously presented): The method of claim 31 which further comprises contacting the washed cells with an *Agrobacterium* eradicant during selection.

Claim 36 (previously presented): The method of claim 35, wherein the eradicant is contained in a layer in or positioned over the gel medium containing the selection agent.

Claims 37-38 (canceled).

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 39 (currently amended): A method for minimizing damage to transformed cells of pine of the genus *Pinus* subgenus *Pinus* following infection by *Agrobacterium* for *Agrobacterium* transformation which comprises:

- (a) washing transformed cells of the subgenus *Pinus* in a liquid culture medium comprising inorganic nutrients, vitamins, amino acids, a carbohydrate source and an *Agrobacterium* eradicator to minimize damage to the cells, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;
- (b) plating said cells on a support membrane;
- (c) suspending said cells in a the liquid culture medium; and
- (d) recovering washed cells with minimal physical damage.

Claim 40 (original): The method of claim 39, wherein (i) cells are plated onto a support membrane and (ii) said cells are transformed prior to step (a).

Claim 41 (canceled).

Claim 42 (previously presented): The method of claim 39, wherein steps (b) and (c) are repeated between 2 and 10 times.

Claim 43 (previously presented): The method of claim 39 wherein each wash is carried out for a duration sufficient to expose all the cells to the liquid culture medium, said wash carried out for between half an hour to overnight in duration.

Claim 44 (canceled).

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 45 (original): The method of claim 39, wherein said support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claims 46-81 (canceled).

Claim 82 (previously presented): The method of claim 2, wherein the support membrane containing the pine cells is placed over a gel medium for transformation, *Agrobacterium* is added to the pine cells on the support membrane and the pine cells and *Agrobacterium* are co-cultivated to produce transformed pine cells.

Claim 83 (currently amended): The method of claim 82, wherein the gel medium for transformation comprises polyethylene glycol (PEG) and a carbohydrate source that is maltose and polyethylene glycol (PEG).

Claim 84 (previously presented): The method of claim 83, wherein the gel medium for transformation comprises 6% maltose and 7% PEG.

Claim 85 (previously presented): The method of claim 5, wherein the support membrane containing the pine cells is placed over a gel medium for transformation, *Agrobacterium* is added to the pine cells on the support membrane and the pine cells and *Agrobacterium* are co-cultivated to produce transformed pine cells.

Claim 86 (currently amended): The method of claim 85, wherein the gel medium for transformation comprises polyethylene glycol (PEG) and a carbohydrate source that is maltose and polyethylene glycol (PEG).

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 87 (previously presented): The method of claim 86, wherein the gel medium for transformation comprises 6% maltose and 7% PEG.

Claim 88 (previously presented): The method of claim 27, wherein the support membrane containing the pine cells is placed over a gel medium for transformation, *Agrobacterium* is added to the pine cells on the support membrane and the pine cells and *Agrobacterium* are co-cultivated to produce transformed pine cells.

Claim 89 (currently amended): The method of claim 88, wherein the gel medium for transformation comprises Polyethylene glycol (PEG) and a carbohydrate source that is maltose and Polyethylene glycol (PEG).

Claim 90 (previously presented): The method of claim 89, wherein the gel medium for transformation comprises 6% maltose and 7% PEG.

Claim 91 (previously presented): The method of claim 32, wherein the support membrane containing the pine cells is placed over a gel medium for transformation, *Agrobacterium* is added to the pine cells on the support membrane and the pine cells and *Agrobacterium* are co-cultivated to produce transformed pine cells.

Claim 92 (currently amended): The method of claim 91, wherein the gel medium for transformation comprises Polyethylene glycol (PEG) and a carbohydrate source that is maltose and Polyethylene glycol (PEG).

Claim 93 (previously presented): The method of claim 92, wherein the gel medium for transformation comprises 6% maltose and 7% PEG.

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 94 (previously presented): The method of claim 13, wherein the gel medium for selection further comprises abscisic acid (ABA).

Claim 95 (previously presented): The method of claim 14, wherein the layer for selection further comprises abscisic acid (ABA).

Claim 96 (previously presented): The method of claim 25, wherein said selection agent is contained in said gel medium.

Claim 97 (previously presented): The method of claim 25, wherein said selection agent is contained in a layer and said support membrane is placed over said layer which is placed on said gel medium.

Claim 98 (previously presented): The method of claim 97, wherein said layer is a layer of liquid medium.

Claim 99 (previously presented): The method of claim 97, wherein said layer is a layer of gelled medium.

Claim 100 (previously presented): The method of claim 97, wherein said layer is a filter paper with a liquid medium absorbed therein.

Claim 101 (previously presented): The method of claim 96, wherein the gel medium for selection further comprises abscisic acid (ABA).

Claim 102 (previously presented): The method of claim 97, wherein the layer for selection further comprises abscisic acid (ABA).

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 103 (previously presented): A method for regenerating transgenic plants of pine of the genus *Pinus* subgenus *Pinus* which comprises:

transforming pine cells of the subgenus *Pinus* with *Agrobacterium* by plating the pine cells on a support membrane, placing the support membrane containing the pine cells over a gel medium for transformation, adding *Agrobacterium* to the pine cells on the support membrane and co-cultivating the pine cells and *Agrobacterium* to produce transformed pine cells;

minimizing damage to transformed pine cells subsequent to *Agrobacterium* transformation by washing cells with a liquid culture medium comprising inorganic nutrients, vitamins, amino acids, a carbohydrate source and an *Agrobacterium* eradicant, wherein said damage is physical damage to the cells and loss of the cells and wherein minimized damage is assessed by time period to regain pre-transformation growth rate;

selecting transformed cells by plating washed transformed cells with minimized damage on a support membrane, placing the support membrane containing the washed transformed pine cells over a gel medium, contacting the cells with a selection agent and selecting transformed cells;

culturing the selected transformed cells to produce transgenic somatic embryos; and
germinating the transgenic somatic embryos to produce transgenic plants.

Claim 104 (previously presented): The method of claim 103, wherein the damage to cells is minimized by:

- (a) suspending the transformed cells in the liquid culture medium;
- (b) agitating the liquid culture medium containing suspended cells to wash the cells and remove *Agrobacterium*; and
- (c) recovering washed cells with minimal damage.

Claim 105 (previously presented): The method of claim 103, wherein the damage to cells is minimized by:

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

- (a) rinsing the transformed cells using the liquid culture medium to remove *Agrobacterium*;
- and
- (b) recovering washed cells with minimal damage.

Claim 106 (previously presented): The method of claim 105, wherein steps (a) and (b) are repeated between 2 and 10 times.

Claim 107 (previously presented): The method of claim 105, wherein each wash is carried out for a duration sufficient to expose all the cells to the liquid culture medium, the wash carried out for between half an hour to overnight in duration.

Claim 108 (previously presented): The method of claim 103, wherein the support membrane is prepared from a material selected from the group consisting of polyester, polypropylene and a liquid permeable fluoropolymer fabric.

Claim 109 (previously presented): The method of claim 103, wherein the selection agent is contained in the gel medium for selection.

Claim 110 (previously presented): The method of claim 103, wherein the selection agent is contained in a layer and the support membrane is placed over the layer which is placed on the gel medium for selection.

Claim 111 (previously presented): The method of claim 109, wherein the layer is a layer of liquid medium.

Claim 112 (previously presented): The method of claim 109, wherein the layer is a layer of gel medium.

Application No.: 09/973,088

Amendment Dated 29 May 2006 filed with RCE

Reply to Office Action of 29 December 2005 and Advisory Action mailed 18 May 2006

Claim 113 (previously presented): The method of claim 109, wherein the layer is a filter paper with a liquid medium absorbed therein.

Claim 114 (previously presented): The method of claim 103 which further comprises contacting the washed cells with an *Agrobacterium* eradicant during selection.

Claim 115 (previously presented): The method of claim 114, wherein the eradicant is contained in a layer in or positioned over the gel medium containing the selection agent.

Claim 116 (currently amended): The method of claim 103, wherein the gel medium for transformation comprises Polyethylene glycol (PEG) and a carbohydrate source that is maltose and Polyethylene glycol (PEG).

Claim 117 (previously presented): The method of claim 116, wherein the gel medium for transformation comprises 6% maltose and 7% PEG.

Claim 118 (previously presented): The method of claim 109, wherein the gel medium for selection further comprises abscisic acid (ABA).

Claim 119 (previously presented): The method of claim 110, wherein the layer for selection further comprises abscisic acid (ABA).